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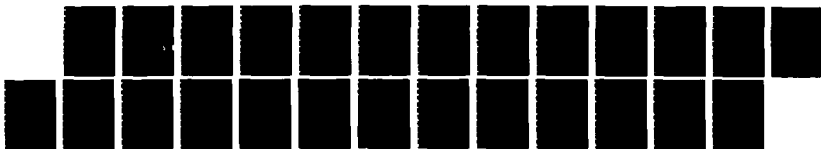
STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY
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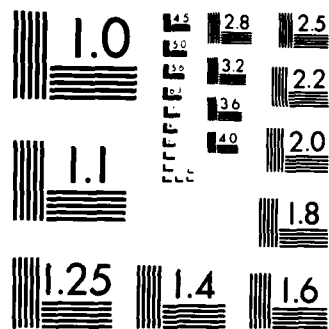
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STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY THERMAL INJURY

ANNUAL PROGRESS REPORT

Carol L. Miller, Ph.D.

May 3, 1983

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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ARMY CONTRACT ANNUAL REPORT

Contract # DAMD 17-82-C-2081

May 3, 1983

INTRODUCTION

The high incidence of fatal septicemia associated with severe thermal injury is believed to result from loss of immunocompetence. This laboratory has been able to identify those burn patients who are at greatest risk for developing fatal sepsis by detecting the loss of certain immune functions by cells of these patients. Besides designing assays to monitor the critical burn induced immunodefects, our experiments have focused on those mechanisms which when triggered by severe thermal injury, can lead to cellular immune aberrations. Direct burn-induced immune dysfunction can result from aberrations in any of the three general types of leukocytes which cooperatively mediate the generation of immune function. These three leukocyte subpopulations are the antigen specific bone marrow derived (B) cell, the antigen specific thymus-derived (T) cell, and a third extremely heterogenous population of leukocytes - the monocyte or macrophage (MØ).

During this contract year our focal point has been both studying burn-induced alterations in monocyte (MØ) function and examination of possible therapeutic modalities designed to reverse or diminish these MØ defects. We have initiated two new MØ assays (measurement of endogenous pyrogen (EP) and complement components) as well as refining the PGE₂ assay which was introduced last year. Our working hypothesis has been that critical alterations in MØ function occur very early in the post-burn period. These early changes in MØ activities unbalance the immune network away from immunocompetence and toward excessive regulation and hypoimmunity.

The monocyte population appears to be divided into facilitory and inhibitory subsets just as the T lymphocyte population is segregated into helper and suppressor cells. A complex reciprocal



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interaction occurs between facilitory MØ (fac MØ) and T helper cells. Recent data indicates that a similar reciprocal interchange occurs between inhibitory MØ and suppressor T cells.

If thermal injury can interrupt and/or alter certain pivotal MØ functions, both PMN function and immune function will be disrupted. This laboratory has been monitoring a number of MØ functions which are critically involved in host defense. The monocyte functions, as well as the immune functions of thermally injured patients are assessed every 3 days from admission to release or demise. Alterations in these MØ activities are determined by comparison to the patients' own initial MØ activity level and to the established "normal" level. The patients' monocytes are routinely monitored for their production of plasminogen activator (PA), tissue procoagulant factor (TF) and lysozyme (Ly). Since October of last year, we have also been routinely monitoring all severe burn patients' MØ for PGE₂ production. In addition, we have now begun to monitor selected burn patients' MØ (those identified as at high risk by mitogen assays) for their production of endogenous pyrogen and complement component 2 (C2). Both of these MØ products are crucial to the inflammatory response. There is also evidence that these two products may have major effects on the functions of specific immune cells. In addition, we have begun developing experiments designed to test various drug therapies for their efficacy in restoring what appear to be critical MØ aberrations. This contract period has seen considerable advancement not only in monitoring burn patients cellular defects but also in defining the critical mechanisms of burn induced immunodefects and in examining several therapeutic modalities. This years' work has been reported in two papers and six abstracts.

METHODS

Human Studies

Patients with greater than 30% full thickness burns are the primary donors of abnormal leukocytes. Normal volunteers are donors of control human leukocytes. Appropriate safety precautions are always observed. Minors, prisoners, pregnant women and the mentally handicapped are excluded as donors. Mononuclear cells are isolated

from the peripheral blood (PB) by Ficoll-Hypaque gradient centrifugation. Patient mononuclear populations can be further depleted of T cells, monocytes and/or B cells. The T lymphocytes are depleted by removing the cells binding to neuraminidase treated sheep red blood cells (SRBC) on a Ficoll-Hypaque gradient. We also are now utilizing monoclonal antibodies (OKT4 and OKT8) to select for human T cell subpopulations. The suppressor/cytotoxic subpopulation is separated from the helper/inducer subset in one of two ways. In one set of experiments, we negatively select by lysing one subpopulation with antibody plus complement (C). In the other set of experiments, we positively select by treating the cells with fluorescenated antibody and sorting out the labeled subset on the fluorescenated activated cell sorter (FACS). Monocytes are removed by passing the mononuclear population over Sephadex G-10 columns. Monoclonal anti-MØ antibodies (OKM1 and OKM5) are also used to both positively and negatively sort monocytes from other mononuclear cells. The B cells can be removed by nylon wool filtrations of the cell population.

We monitor the ability of patient and normal mononuclear cell populations to respond to phytohemagglutinin (PHA). This non-specific mitogen response requires the cooperative interaction of monocytes and T cells.

Monocytes (MØ) are isolated from the Ficoll-Hypaque purified mononuclear cell populations by the Ackerman and Douglas rapid adherence technique. These isolated monocytes are then routinely examined for the production of PA, their level of tissue procoagulant factor (TF), their generation of prostaglandin E₂ (PGE₂), and their synthesis of lysozyme. MØ from selected patients are also assayed for their production of endogenous pyrogen (EP) and C2. In the PA assay, patients' and normal controls isolated MØ are placed into ¹²⁵I-fibrin plates and cultured 18 hours either in the presence of acid treated fetal bovine sera (AT-FBS) or soybean trypsin inhibitor (SBI), an inhibitor of plasmin. After all the PA is released in these cultures, the cells are washed and fresh AT-FBS media or SBI media are added for an additional 24 hour incubation period. The amount of fibrinolysis initiated during this second

incubation period is then measured. Monocyte numbers have been adjusted to produce approximately 25-35 fibrinolytic units for normal individuals (4×10^5 isolated MØ). Simultaneous to our assessment of burn patients' monocyte's PA synthesis, we also assay their production of TF and lysozyme. TF production is measured using the Rickle's assay and lysozyme production is measured during the Schill and Schumacher Lysozyme Plate test.

Samples used in the PGE_2 assay are obtained from the MØ supernates of burn and trauma victims. Mononuclear cells are separated from peripheral blood by ficoll-hypaque centrifugation. The cells are incubated on flasks (8×10^6 cells/ml) for $1\frac{1}{2}$ hours to select for the monocyte population. Fresh media is put on the flask and the cells are incubated overnight. The media and the adhered cells are removed from the flask and cell counts are taken. The media is aliquoted into 2 ml samples which are stored at -85°C until extraction.

The extraction procedure being used now is petroleum ether mixed gently (3X) in equal amounts with the sample. This helps to remove lipids which interfere with the accuracy of the assay.

In the near future, chromatography columns will be employed to clean up the sample further prior to assay. We are investigating the use of the Sep-pac C18 extractant column from Waters Associates, Milford, MA.

Radioimmunoassay: Standards of the following concentrations are used for a standard curve for sample comparison; 25 pg/.1 ml, 10 pg/.1 ml, 5 pg/.1 ml, 2.5 pg/.1 ml, 1.0 pg/.1 ml, 0.5 pg/.1 ml, 0.25 pg/.1 ml, "0" pg/.1 ml.

100 μl of the standards and the samples are aliquoted into the corresponding polypropylene tubes. 100 μl of PGE_2 (I^{125}) tracer is added. 100 μl of rabbit PGE_2 antibody is added and the tubes are vortexed. Tubes are incubated overnight at $2-8^\circ \text{C}$.

After 16-24 hours, tubes are put on ice and 1 ml of cold precipitating reagent is added. Tubes are vortexed and incubated on ice for 20-30 minutes. After incubation, tubes are centrifuged at 2500 rpm at $2-8^\circ \text{C}$ for 30 minutes. Supernates are decanted and

residue is counted on a gamma counter for 1 minute count time. Counts are then compared to the standard curve for PGE₂ concentrations.

The cell free supernates collected from the MO during isolation of the Ackerman Douglas flasks can be assessed for EP as well as for PGE₂. These supernates are assessed by using a minor modification of the method previously described by Bodel and Miller. Briefly, 12 weeks old male Balb/C mice are prewarmed at a slightly higher temperature of 38-40° C. After 1 hour, they are removed every 10 minutes for rectal temperature readings, taken by means of a thermistor probe (Yellow Springs Instrument Co., Yellow Springs, Ohio), inserted to a distance of 2 cm for 1 minute. After 50 minutes, stable baseline temperatures are achieved, at levels between 36.5° C and 38° C. These mice were then injected intravenously with 0.3 ml of test patients' supernates. Temperatures of the mice are monitored every 10 minutes thereafter for 50 minutes. Individual mice receive no more than three injections over a three week period before they are sacrificed.

When a patients' MO are to be examined in the complement assay, additional Ackerman Douglas flasks are prepared as described above. The flasks are separated into different groups; unstimulated cells (used as a control) and stimulated cells with Fc fragments (50 mg/ml), PHA (1 mg/ml) and PPD (10 mg/ml). The cells are cultured for two days or four days and then assessed in the complement assay. The synthesis of the C2 complement component by the monocyte is measured by the hemolytic plaque assay as developed by Colten. Briefly, the cell suspension from the cultured flasks (at 4×10^6 /ml) are mixed with stable cellular intermediates EAC₁₄ (Cordis Lab) at 10^9 /ml, and with 0.5% agarose then left at 47° C in a water bath. This mixture is poured on a coated slide. These slides are incubated for about one hour at 37°C. Hemolytic plaques appear after 40 minutes incubation at 37° C with 1/40 EDTA treated rat complement.

Utilizing our present assay system, from one 20 ml blood sample, we can simultaneously measure PHA, MO PA generation, MO PGE₂ and EP synthesis, MO lysozyme and TF production and MO

complement synthesis. To detect suppression we need to draw additional blood samples. Human mononuclear cells are separated into T, B or MØ subpopulations and the interaction between these subpopulations is evaluated. The effect of suppressor T cells or of inhibitory MØ is assessed by mixing purified autologous cell populations and assessing the affect of one cell type (i.e. suppressor T cell) on another cell populations' function.

A new assay for measuring burn-induced suppression has been developed by this laboratory. This assay assesses the ability of burn patients' E-rosette positive T cells and/or MØ to suppress PA production by a normal individuals' MØ. The burn-induced suppressive cells are incubated 24 hours with isolated normal controls' MØ, the normal MØ's are re-isolated, adjusted to 4×10^5 /well and assayed in our PA system as described above. We compare the PA production of MØ incubated with allogeneic burn patients' cells to their PA production after exposure to allogeneic normal individuals' cells.

We have detected burn-induced aberrations in the immune regulation functions of patients' cells using a modification of the classical mixed lymphocyte response (MLR). In our MLR system, a highly responsive combination of cells from two normal individuals is cultured in a "one way" MLR. In this assay, one group of the normal's cells are pretreated with mitomycin C (MC) to prevent their division. Consequently, this "one way" MLR assay measures the ability of one group's normal cells (Responder=R) to proliferate in response to the foreign histocompatibility antigens on another normal's cells (Stimulator=S). We compare the effect of adding either burn patient cells or MC treated responder cells on the amount of proliferation measured in the MLR cultures.

Data Calculation and Statistical Analysis

The data presented for patient and normal's PA production is always from the second incubation interval. All supernate CPM's of ^{125}I -fibrin are corrected for media and non-specific radioactivity release by subtraction of CPM's obtained from the no cell control. The CPM's of ^{125}I -fibrin in the supernates from the lines containing cells in 100 µg SBI are subtracted from the CPM's of lines

containing the cells in AT-FBS. This corrects for any ^{125}I -fibrin lysed by any non-plasmin mediated mechanisms. This corrected AT-FBS CPM is then divided by the total ^{125}I -fibrin CPM's present to derive the percent specific plasmin mediated lysis. This value is computed for patient cells collected every four days post-injury. The mean and standard deviation of PA production by MØ from 43 normal individuals tested repeatedly was 25 ± 8.4 . The patient data were calculated by comparing the PA response at various days post-injury to both the normal values (25 ± 8.4), and their own initial (day 1) values. A Student's t-test was used to determine significant differences. The TF activity of sonicates from 10^5 MØ was calculated in thromboplastin equivalent units by comparison of the shortened thromboplastin time to a control brain thromboplastin standard curve.

Calculation of PGE_2 in sample

Normalization of the standards and the samples is as follows:

% B/Bo vs Pg of PGE_2 is graphed on semi-log paper.

Standard or sample counts minus counts from background "blank" sample = net counts.

% B/Bo = $\frac{\text{net counts standards or samples}}{\text{net counts "O" standard}} \times 100$

% B/Bo standards are graphed and %B/Bo samples are compared to determine pg of PGE_2 .

Pg of PGE_2 is per 100 μl so this is adjusted to $\text{Pg}/10^6$ cells in the following manner:

$$\frac{\text{Pg of } \text{PGE}_2}{100 \text{ ul}} \times \frac{1000 \text{ ul}}{1 \text{ ml}} \times \frac{(\text{dil factor})}{1} \times \frac{\text{\#ml Patient sample}}{\text{cell count of adhered cells}}$$

For high concentrations of PGE_2 (25 pg/.1 ml) dilutions of the original sample must be done and accounted for in the calculations.

The PGE_2 assay requires extensive data processing as described under Methods. We have written a computer program to handle this data. Unfortunately, the Hewlett Packard computer we are sharing time on has become almost inaccessible. Not only do we use it for the PGE_2 data but also to process the PA and lysozyme data.

Presently, we face delays on data calculation of up to one week. As requested under separate cover, we would like to purchase a micro-computer system for this laboratory.

Human peripheral blood mononuclear cell populations differ from individual to individual in their percentage of MØ, T and B cells and their degree of immune reactivity. It has been suggested that human immune functions are controlled by immune response genes analogous to those described in animal systems. Consequently, the "normal" levels of MØ PA production, mitogen responsiveness, MØ TF generation, lysozyme production, and MØ PGE₂ activity vary for each patient and within the normal control groups. The baseline levels of each individual's MØ and T cell activities are not randomly distributed. Some individuals are low and some are high responders. This nonbinominal distribution of the MØ and T cell parameters necessitates the use of non-parametric statistics when analyzing patients' data. We utilize the Wilcoxon test for evaluating the statistical significance alterations in patients' mitogen, PA, and TF assays. We utilize Spearman's correlation coefficient for determining the degree of interdependence between the various MØ and T cell parameters.

Guinea pig experiments

70 strain 13 inbred guinea pigs of both sexes 300-400 grams in weight were used in these experiments. The monocytes' dependency of the guinea pig immune response is much greater than that seen with the murine system. An in vitro prime is therefore necessary in order to measure an in vitro immune response. The guinea pig in vitro secondary response (like the human system) is much more subject to disruption than the mouse. Consequently, the guinea pig is a more comparable model for evaluating immune dysfunctions in thermally injured patients.

A primary challenge is administered by subcutaneous injection of 0.3 ml complete Freund's Adjuvant (Gibco) and 2% sheep red blood cells (Gibco) emulsion in the the footpad. On day six after the primary challenge, some of the guinea pigs are anesthetized with 3.5 mg/kg body weight Ketamine and receive a 20-30% total body

surface area third degree scald burn (90° C 30 sec). The sham injured guinea pigs were anesthetized but not burned.

Three injections of TP5 were given. At four to six hours, 24 hours, and 48 hours post-burn one group of the guinea pigs received an IV injection of 1 mg/kg TP5 (Thymopentin, Ortho Pharmaceutical Corporation). In later experiments, the TP5 dosage was increased to 3 mg/kg body weight. Finally, 3 mg/kg TP5 was injected IV in combination with 1.5 mg/kg Indomethacin (Sigma) administered intraperitoneal. A second group of burned guinea pigs along with the sham injured guinea pigs received equivalent IV injections of saline.

At day four post-burn, the guinea pigs were sacrificed by CO₂ asphyxiation and the spleens sterilely removed. The in vitro generation of antibody forming cells has been previously described.

The number of antibody forming cells (AFC) to sheep red blood cells (SRBC) is assayed using the slide modification of hemolytic plaque assay. Plaques are visually counted.

Spleen cells were maintained five days in a modified Mishell-Dutton culture system. The Mishell-Dutton system was modified as follows: Six-well tissue culture plates were used to culture 1.2×10^7 cells in 1 ml Iscoves liquid media supplemented with a final concentration of 1% Penicillin/Streptomycin, 1% L-Glutamine, 0.5% L-Asparagine, (all Irvine Scientific), 1% Garamycin (Schering), 1% Trypticase Soybroth, 5×10^{-5} M 2-Mercapto-ethanol and 4% heat inactivated Rabbit Serum, (Kappa Scientific). 50 ml of one percent SRBC were used as immunogens in test wells, each test well had a control well with SRBC.

The in vitro generation of AFC is assayed using the slide modification of the Hemolytic Plaque assay. Leukocyte recovery from cultures is determined by counting a sample of the harvested, cultured cells on a Coulter Counter (Model ZH). The number of AFC are calculated for each pool of duplicate background plaques and expressed as AFC/ 10^6 recovered spleen cells. Allogeneic conditioned media is produced as described.

RESULTS AND DISCUSSION

This contract year we have monitored post-burn alterations in the host defense systems of eighteen severely burned patients. These eighteen severely burned individuals ($> 30\%$, 3^0) were studied every four days post-injury from admission. Of this group, 7 in critical succumbed to overwhelming sepsis. We divide our patients into 3 groups on the basis of their mitogen responses. Group I patients' mononuclear cells show less than a 33% change in mitogen responsiveness during their recovery period. These patients also have an uneventful clinical course. Group II patients' exhibit a hypermitogen response usually in response to a infectious episode. This mitogen hyperimmunity is typical of the normal immune system dealing with an infectious challenge. This hyperimmune response usually occurs at 10 to 14 days post-injury. These Group II patients have a clinical course characterized by an infectious episode which they successfully overcome. The Group III patients show a profound and early (5-7 days post-injury) depression of their mitogen responses. Their clinical course is punctuated with repeated septic episodes from which the patient often succumbs. Table 1 illustrates the main data collected on this year's patients by thermal injury. As can be seen, the depression of the mitogen response is always accompanied by a depression in MØ function as measured by the production of plasminogen activator (PA). This decrease in MØ PA production is not accompanied by cessation of all MØ functions. Lysozyme production is either unchanged or increased in these patients. These data indicate that MØ functions are selectively effected. As can be seen in Table 1, the MØ PA response is more sensitive to burn induced alterations than the PHA response. All of the severely thermally injured show a decrease in PA response and this depression appears earlier (2-3 days post-injury) and persists even when mitogen responses have returned to normal. These data are consistent with our hypothesis that severe thermal injury mediates earlier changes in critical MØ function. If these changes in pivotal MØ functions are drastic enough, immune function and other host defense systems such as neutrophil chemotaxis and phagocytosis are critically depressed.

Another experimental design we are using tests our hypothesis that early MØ alterations are pivotal in overall depression of host defense, involves monitoring MØ PGE₂ production. Excessive PGE₂ levels can directly suppress MØ function, lymphocyte function, and PWM maturation while increasing T_s generation. Consequently, an increase in PGE₂ levels could be a primary trigger of many of the alterations seen post-burn. If elevated PGE₂ levels are the major contributors to post-burn immunodepression, then specific treatments, like indomethacin which is antagonistic to PGE₂ synthesis might reverse some or all of the post-burn immunodepression. Experiments examining indomethacin in an animal model are described in a later section. In our current patient assays, we have attempted to establish that an increase in MØ PGE₂ does occur in severe thermal injury and that this increase in MØ PGE₂ production correlates to clinical outcome. Table 2 illustrates data on PGE₂ levels in some of the patients studied this year. We have encountered some difficulty with the RIA kit we were initially using to measure PGE₂ levels in the MØ supernates. This kit requires an extensive extraction and then a conversion of PGE₂ to PGB. Both of these procedures have low efficiency and a highly variable product recovery. The consequences of these technique problems are that our accuracy in quantitating PGE₂ amounts was poor. When we ran different known quantities of PGE₂ through our assay system, we found that we could not detect amounts less than 15,000 pg and that we could not discriminate 50,000 pg from 100,000 pg. This means that when we detect 40,000 pg in patient samples using the ³H-PGE₂ kit, the actual PGE₂ levels are much higher. This insensitivity is probably why patient PGE₂ production seemed to appear and disappear rather than progressively increasing and decreasing. We are now using a commercial RIA kit which detects PGE₂ directly (no conversion necessary) and requires only a column extraction rather than an ether lipid extraction. The columns for this extraction are commercially available and the whole procedure can now be completed on the same day. We expect that this improved assay should allow us to detect more subtle differences in MØ PGE₂ production post-burn.

Alterations in M ϕ PGE₂ production may also be affecting M ϕ production of EP. EP and Interleukin 1 (IL-1) appear to be two different activities of the same biological moiety. PGE₂ is known to depress IL-1 production. Alterations in EP levels have been implicated in post-burn metabolic changes, as well as in changes in immune function. We had some initial technical difficulties utilizing the EP assay. In order to obtain stable baselines, it was necessary to warm the mice to 39° C and to increase the size of animals employed. We then had to establish the baseline response for supernates from our normal controls. As can be seen in Table 4, control M ϕ increased the mouse temperature from 0 to .3° C. Simultaneously run patients' M ϕ supernates ran from a negative value (-0.2) to a high of 0.83. Group II burn patients (who experienced infectious episodes but not sepsis) have massive increases in EP levels. This hyper M ϕ response may be similar to the hyper mitogen responses that we have characterized in Group III patients. As the data in Table 3 illustrates, Group II patients (who are immunocompromised) experience a decrease in EP production at day 2-6 post injury. Several patients M ϕ supernates actually caused a reproducible decrease in the temperature of the mice. We are investigating these supernates to determine if they actually contain some factor antagonistic to EP.

The depressions we have so far detected in M ϕ EP production are not as dramatic as the reduction in M ϕ complement synthesis that occurs after severe burns. M ϕ synthesis of some of the C components (C4, C3, C2, C5, Bb) controls their concentrations at the local injury site. Consequently, a decrease in M ϕ synthesis of various critical C components could lead to insufficient C levels at the injury site even though no decrease in serum complement levels was detected. We have monitored the level of C2 synthesized spontaneously and after in vitro stimulus with antibody fragments or lymphokines. The data in Table 4 represents the number of M ϕ synthesis, C2 per 10⁶ total M ϕ . As can be seen, there is a significant difference between the synthesis activity of normal and hypo-mitogen responsive burn patients. This difference in synthetic ability is even greater when stimulated M ϕ population of control

and burn patients are compared. After severe thermal injury, the MØ at the injury site should be activated to increase C synthesis. In fact, burn patient MØ are unable to respond to immune stimuli with increased MØ C synthesis. Consequently, our data imply that an immunoincompetent burn patient would not only have reduced C levels because of decreased lymphokine activity, but also that the MØ themselves would have reduced synthetic capacity. The level of fresh C available at the injury site for PMN chemotaxis and phagocytosis would be drastically reduced for these patients. We have only begun to monitor burn patients MØ complement synthesis. At present, we are only assaying those patients whose MØ PA production falls early (1 - 3 days) in the post-injury period. In order to perform the C assay, we must draw more than our usual 15-20 ml of blood. Consequently, we are currently only assessing the patient once during the period of maximal hyporesponsiveness (5-7 days post-burn). We also spent time establishing what the range of C synthesis was for normal individuals. However, the initial data from the complement assay have convinced us that increased monitoring of the patient C synthesis will yield vital information on patient immune status. One of the important correlations we will be examining is if increased PGE₂ production can depress MØ C synthesis. In addition, we expect to expand our assay system to measurement of C4 and C3. The hemolytic plaque assay as originally described was for C2. However, Colten and colleagues have recently published modified and improved methods for measuring the other C components as well. We hope to initiate the new improved assay during this next year. Our C data appear to support our hypothesis that thermal injury mediates changes in MØ functions and that these changes are pivotal in post-burn depression of host defense.

Decreased MØ function and augmented regulatory activity seem to be the key defects in the immunoincompetent burn patient syndrome. Consequently, prophylactic therapy which is directed toward decreasing T_s and/or moderating PGE₂ activity should benefit burn victims. Utilizing our guinea pig (g.p.) burn model we have exa-

mined in vivo injection of TP5 (a thymopoetin pentapeptide) and indomethacin (a PGE₂ antagonist) for their modulation of the decreased antibody forming cell (AFC) response in our burned g.p. system. The experimental design was as follows: all male or all female, syngeneic age matched g.p. of either strain 2 or strain 13 were divided into 3 groups. One group was sham injured as previously described and served as controls for the AFC response. The second group was thermally injured and then their splenocytes were assayed in the AFC response. The third group was burned and initially injected with either 1 mg/kg/day of TP5 or 3 mg/kg/day 18 hr post-injury and then received 2 subsequent injections on each of the next 2 days. The three groups were assayed simultaneously. As can be seen in Table 5, the response of the burned g.p. group was markedly reduced from that of the control group simultaneously burned. The animals who received 1 mg/kg/day of TP5 for 3 days post-burn showed a significant increase ($p < .005$) in their AFC response over the burned group but still did not exhibit complete restoration to control response levels.

In initial experiments, the difference between the 1 mg/kg dose and the 3 mg/kg dose were not significant. However, with further testing the 1 mg/kg dose appeared more effective $p = .05$. In another set of experiments, we examined in vivo administration of indomethacin as a prophylactic therapy. Indomethacin should prevent increased $\text{inh M}\phi$ synthesis of PGE₂ after burns. Again, the animals were divided into 3 groups, 2 burned and one control. One of the burn groups received indomethacin. The data (Table 6) supported the conclusion that indomethacin could partially restore the AFC response ($x = 40.5\% \pm 7.5$ of control). It is of particular interest that neither TP5 nor indomethacin by themselves could totally restore the AFC response after severe thermal insult. The therapeutic action of TP5 is directed at expanding the T_h population thereby moderating to some extent the depressive effect of T_s . In contrast, the action of indomethacin is to prevent excessive PGE₂ synthesis. In this case the target of the drug is presumably the $\text{inh M}\phi$. Since neither treatment by itself was completely effective in restoring the AFC response, it suggests that at least two

separate immune defects are generated by burns. In a preliminary set of experiments, we have examined the effect of combining both TP5 and indomethacin treatment in our burned g.p. model. As illustrated in Figure 1, combination of TP5 and indomethacin appeared to be slightly more effective in restoring immune function than either drug by itself. In our present studies, we are examining this combinational therapy and using various different levels of TP5 and indomethacin. It is possible that lower doses of both drugs would be more effective in combination, than the higher levels we employed when testing each drug alone.

This year has been particularly productive. We have developed the MØ C assay, and have accumulated data indicating that this new assay is monitoring an important burn altered MØ function. We are improving and refining the PGE₂ assay while gathering important new indications of the inimical effects of post-burn elevation of MØ PGE₂ on immune function. We also now have the capacity to identify human T_s subsets using the fluorescent cell sorter and expect to expand this capacity in the coming year. Most important, we are now beginning to examine various prophylactic treatments for their efficacy in reversing a number of burn mediated immune defects. We expect even more interesting data and several publications to result from experiments now in progress.

T A B L E 1

<u>Patient</u>	<u>Maximum % PA Suppression</u>	<u>Maximum % PHA Variation</u>	<u>Outcome</u>
<u>GROUP I</u>			
AR	25	+25	No complications released
PH	14	+38	No complications released
FA	33	-19	No complications released
<u>GROUP II</u>			
EL	42	+201	Staph infection recovered
HE	26	+325	Pseudomonas infection recovered
RI	38	+183	Staph infection recovered
TW	41	+210	Staph infection recovered
YI	36	+191	Staph infection recovered
ZY	28	+205	Pseudomonas infection recovered
<u>GROUP III</u>			
KE	85	-93	Succumbed to serratia sepsis
FI	59	-92	Succumbed to staph sepsis
MAI	84	-75	Succumbed to candida sepsis
MAT	58	-90	Succumbed to pseudomonas sepsis
MO	74	-78	Succumbed to staph sepsis
MC	80	-79	Succumbed to pseudomonas sepsis
SH	65	-67	Succumbed to pseudomonas sepsis
SI	61	-82	Staph, pseudomonas, enterococcus sepsis eventually recovered
PI	72	-75	Multiple septic episodes recovered

T A B L E 2

Correlation of PGE_2 with Massive Increase of PGE_2 at 1-4 Days Post-Burn

<u>Patient</u>	<u>Max PGE_2 1-4 Days</u>	<u>Max PGE_2</u>	<u>Outcome</u>
<u>Group I</u>			
AR	+200	+500	No complication released
PH	+4,113	+10,000	No complication released
<u>Group II</u>			
EL	+725	+11,566	Staph infection recovered
RI	+2,254	+34,503	Staph infection recovered
ZY	+1,404	+22,871	Pseudomonas infection recovered
<u>Group III</u>			
MO	+8,270	+8,270	Succumbed to staph sepsis
MC	+48,090	+48,090	Succumbed to pseudomonas sepsis

T A B L E 3

Change in Burn Patients Endogenous Pyrogen over Time Post-Injury

	<u>Group III pt</u>	<u>Group III pt</u>	<u>Group II</u>	<u>Control</u>
Day 1	+0.3	+0.35	0.1	+0.2
Day 4	-0.2	0.05	-	+0.3
Day 7	+0.55	0.33	-	0
Day 10	+0.45	0.57	0.7	0
Day 14	0	0.27	0.83	+0.2
Day 17	0	0.30	0.35	+0.1
Day 21	-	-	0.3	0.2

T A B L E 4

Post Burn Depression of MO Component Synthesis

Plaques/10⁶ MO

	<u>\bar{X} Control</u>	<u>\bar{X} Burn Patient (d 5-7)</u>
Unstimulated	9.1 \pm 1.1	5.0 \pm 0.5
Fc stimulated	20.5 \pm 2.6	9.0 \pm 1.3
PHA stimulated	19.5 \pm 2.2	10.0 \pm 1.4

TABLE 5

Effect of in vivo Administration of TP5 on Burn Induced Suppression of AFC Response

% Sham Injured Control Generated AFC

per 10⁶ Recovered Cells1 Mg/Kg injected x 3

VII

VI

V

IV

III

II

I

5%

10%

<1%

17%

<1%

<1%

Burn

48%

24%

30%

68%

13%

35%

Burn & Inj TP5

3 Mg/Kg injected x 3

6%

10%

7%

<1%

<1%

<1%

4%

Burn

31%

25%

49%

28%

17%

43%

46%

Burn & Inj TP5

T A B L E 6

Effect of In Vivo Administration of Indomethacin in
Burn Induced Suppression of AFC Response

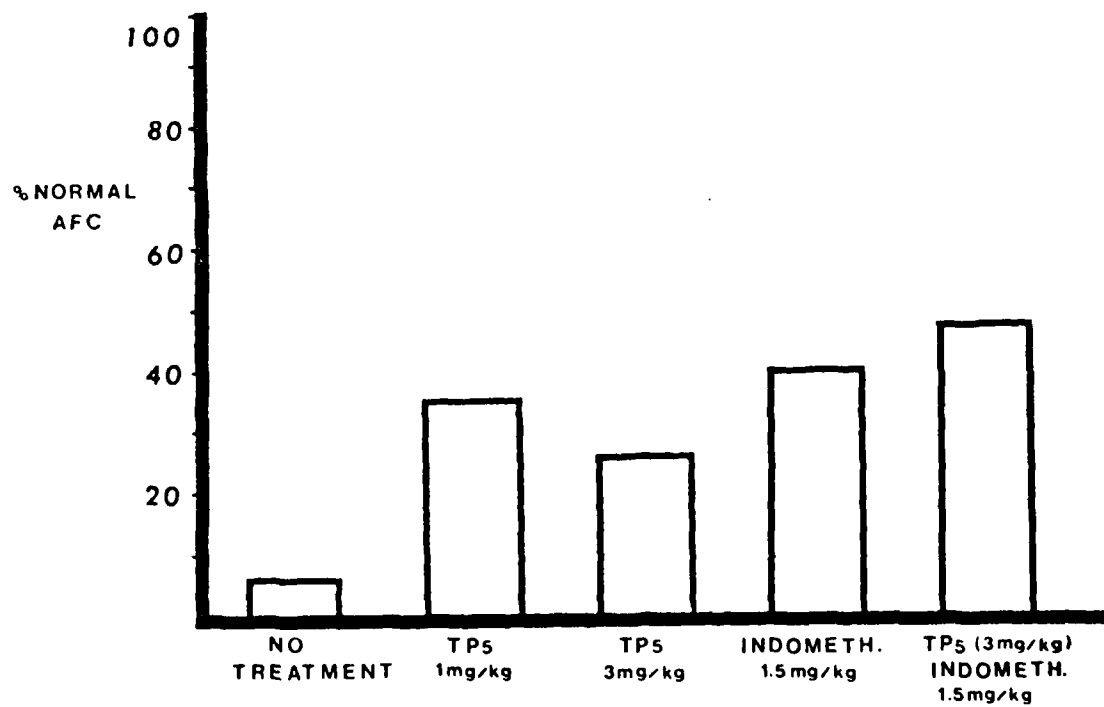
1.5 mg/kg Indomethacin Injected 3x Intraperitoneally Post-Burn

% of control

Burn	6	1	8	3	11	4	21
Indomethacin	44	35	50	38	45	31	

FIGURE 1

EFFECT OF TP5 AND INDOMETHACIN ON BURNS



END

10-86

DT/C